

Safety and Efficacy of Photodynamic Therapy using BCECF-AM Compared to Mitomycin C in Controlling Post-operative Fibrosis in a Rabbit Model of Subscleral Trabeculectomy

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Introduction

- Trabeculectomy is the most frequently applied surgical method to reduce IOP in patients with glaucoma.
- It is generally performed when medical therapy fails to adequately control IOP.
- Excessive subconjunctival scarring following surgery is responsible for failure of the surgery in the majority of cases.



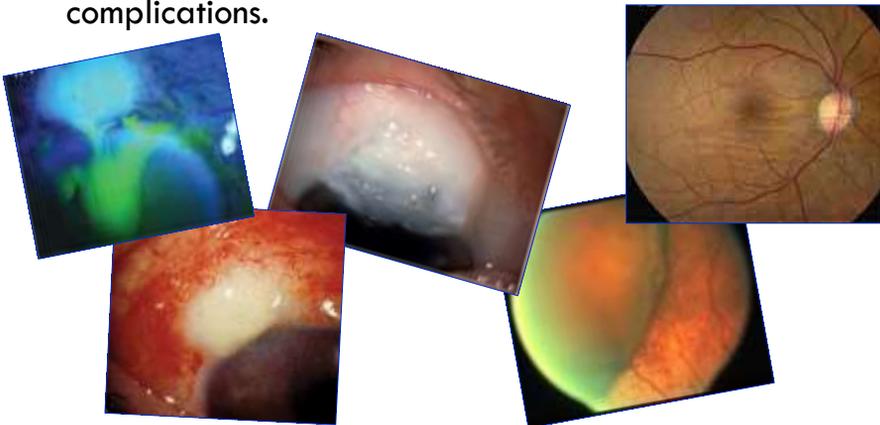
Introduction

- Antimetabolites are commonly used to reduce the formation of scar tissue at the site of surgery and enhancing the long-term success of it.



Introduction

- However they are associated with an increased incidence of severe and potentially blinding complications.

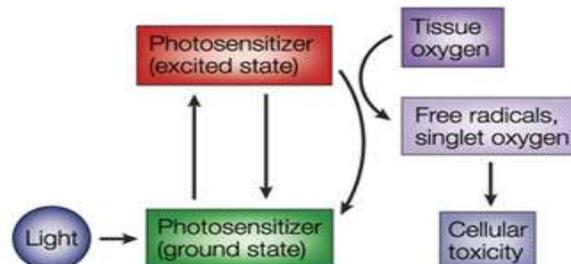


Introduction

- Photodynamic therapy has been used for some ophthalmological diseases such as:
 - **Ocular tumors**
 - **Choroidal and corneal neovascularization**
 - **Proliferative vitreoretinal disorders**

Introduction

- Photosensitisers are used as mediators of light induced cell toxicity.
- They act via the formation of reactive oxygen intermediates and free radicals.
- Selective activation of the photosensitiser by light application at the appropriate wavelength limits the drug effect on the targeted area.



Introduction

- BCECF-AM (carboxyfluorescein derivative) is a cell membrane permeable compound rendered membrane impermeable and fluorescent upon cleavage by intracellular esterases.
- Exposure of cells that have incorporated BCECF-AM to light at the appropriate wavelength leads to cellular photo ablation.



Aim of the work



Aim of the work



To evaluate the safety and efficacy of cellular photoablation using BCECF-AM as a method to control postoperative fibrosis in trabeculectomy compared to the effect of MMC combined with the same procedure in a rabbit model.

Aim of the work

Because of the aggressive wound healing response in rabbits, this animal model is believed to be equivalent to high risk eyes in humans and surgical failure results within one week.



Materials and Methods



Materials and Methods

- A comparative prospective case-control animal study was conducted at Ophthalmology, Pathology and Medical Research Center, Faculty of Medicine, Ain Shams University.



Materials and Methods

- 32 eyes of 16 healthy adult New Zealand white male rabbits.
- The eyes were allocated into one of four groups:
 - **Control group (I):** (4) eyes with **no** surgical intervention.
 - **Control group (II):** (14) left eyes had **SST without** intra-operative administration of **wound modulating agents**.
 - **Study group (I):** (7) right eyes had **SST combined** with intra-operative application of **MMC** (0.3mg/ml).
 - **Study group (II):** (7) right eyes had **SST combined** with intra-operative application of **BCECF-AM** photosensitiser (dose of 80 µg in 300 µl BSS).

Materials and Methods

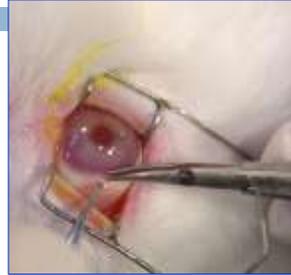
1-Preoperative

- IOP was recorded using hand held Perkin's applanation tonometer.
- To exclude cyclic variations, IOP was compared between the right and left fellow eye pre- and postop.
- The difference in measured IOP was expressed as **right eye/left eye ratio**



Materials and Methods

2-Operations

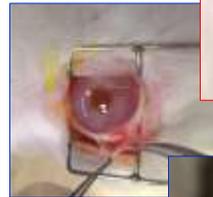


Materials and Methods



Materials and Methods

- A single dose of BCECF-AM (80 μg in 300 μl BSS) was applied subconjunctivally in the region of the proposed filtering bleb.
- The injection was made 10 mm from the corneal limbus by a (27) gauge needle.
- A fornix-based conjunctival flap was performed.
- The episcleral and subconjunctival Tenon's were irradiated for 8 minutes, starting (15) minutes after the injection, with blue light provided by direct ophthalmoscope from a distance of approximately (10) cm.



Materials and Methods

3-Postoperative

- Tobradex eye drops 1X5 for 3weeks.
- All rabbits were examined daily for evaluation of the bleb morphology and detection of any postoperative complications.
- IOP was measured at day 1,3,5,7,14,21 postoperatively.
- Success was defined by
 - **> 20.0 % reduction** in IOP from the preoperative values without anti-glaucoma medications.
 - IOP ratio between the right and left eye **< 0.8**



Materials and Methods



4- Histopathology

- Animals were sacrificed after 3 weeks under general anesthesia.
- Eyes were enucleated, fixed in formaldehyde 10%.
- The specimens were embedded in paraffin and sectioned.
- Sections were stained with
 - Hematoxylin-Eosin → **Cellularity** including fibroblast.
 - Periodic acid - Schiff → **Goblet cell** identification.
 - Masson's Trichrome → **Collagen** density and architecture.
 - Orcein stain → **Elastic fiber** detection.

Results



Results

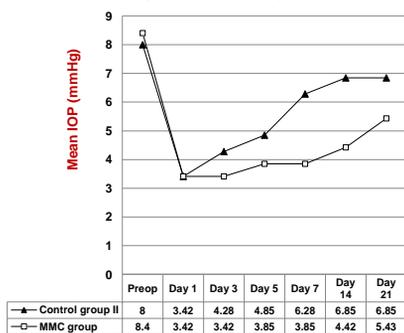
- No post-operative complication was observed.
- Bleb morphology at the end of 3 wks:
 - ▣ In control group (II): small and vascularized.
 - ▣ in the study group (I): elevated and avascular.
 - ▣ In the study group (II): elevated and less vascular than the control group (II) and remained so till the end of the 3 weeks.



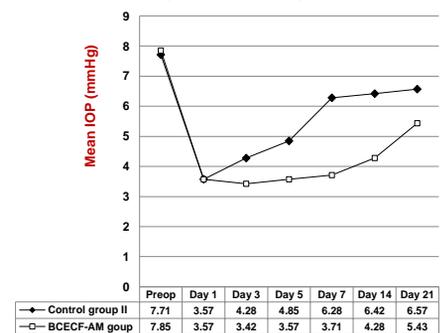
Results

□ Mean IOP

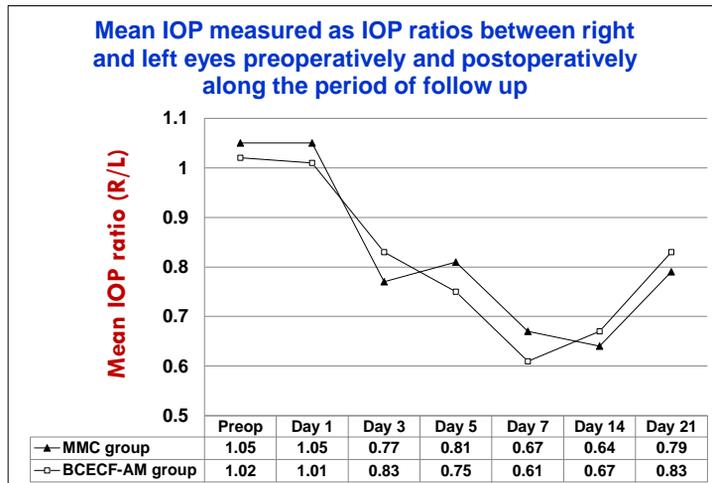
Comparison between the right eye (study group I : MMC) and the left eye (control group II) as regards mean IOP (mmHg) along the period of follow up



Comparison between the right eye (study group II :BCECF-AM) and the left eye (control group II) as regards mean IOP (mmHg) along the period of follow up



Results

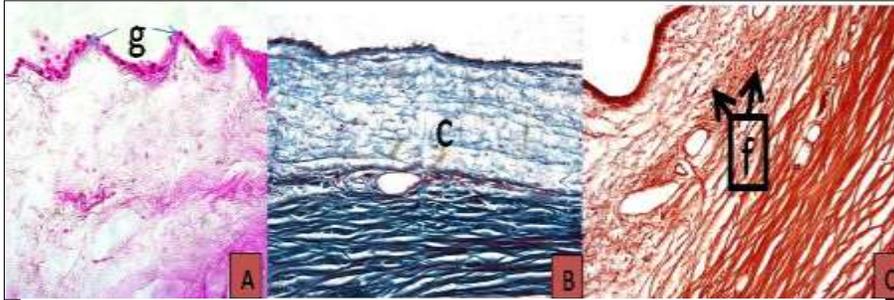


Results

- At the end of three weeks: The mean % of reduction of IOP in the two study groups were > 20.0 % without anti-glaucoma medications.
 - The mean % of reduction was **35.0** in the study group (I) with only one eye (14.3%) had 12.5% reduction.
 - The mean percentage of reduction was **28.0** in the study group (II) with two eyes (28.6%) in study group (II) had 14.2% reduction each.
 - Regarding the control group (II); the mean % of reduction was **14.3** with 5.0/14.0 (35.7%) eyes had > 20.0 % reduction.

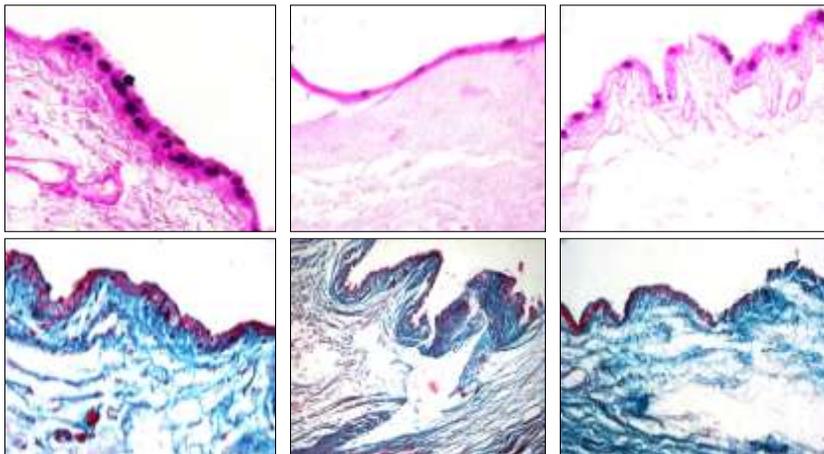
Results

□ Histopathological results



Conjunctiva of the control eyes shows abundant goblet cells (g) normal epithelial thickness and architecture, mild collagen (c) dispersion and scattered black elastic fibers (A: PAS X200, B: MT X200 and Orcein X400).

Results



SST alone

MMC group

BCECF-AM group

Discussion



Discussion



- A completely healed trabeculectomy is a failed trabeculectomy. The use of antifibrotic agents to improve the success of glaucoma surgery has become common practice, and the benefits provided by these agents are accompanied by unique complications.

Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. Surv Ophthalmol 2003; 48:314-346.

Discussion



- To avoid toxic effects on intraocular tissues, it would be valuable to have an agent that will act only on the side of interest. This problem might be solved by cellular photoablation using light-absorbing chemicals.

Grisanti S, Gralla A, Maurer P, Diestelhorst M, Kriegelstein G, Heimann K. Cellular photoablation to control postoperative fibrosis in filtration surgery: in vitro studies. Exp Eye Res 2000;70:145–152.

Discussion



- *Hill et al. 1997* investigated the feasibility of photodynamic therapy in a rabbit model of filtration surgery. Using ethyl etiopurpurin, a photosensitizer traditionally delivered by intravenous injection, they showed that after subconjunctival injections, large areas of avascular conjunctiva were produced and filtering bleb survival was prolonged.

Hill RA, Crean DH, Doiron DR, McDonald T, Liaw LH, Ghosheh F, Hamilton A, Berns MW. Photodynamic therapy for antifibrosis in a rabbit model of filtration surgery. Ophthalmic Surg Lasers 1997; 28:574 - 581.

Discussion



- In vitro, it was shown to be phototoxic for human Tenon fibroblasts which were the major cells types involved in subconjunctival fibrosis and bleb failure after filtration surgery.

Grisanti S, Gralla A, Maurer P, Diestelhorst M, Krieglstein G, Heimann K. Cellular photoablation to control postoperative fibrosis in filtration surgery: in vitro studies. Exp Eye Res 2000; 70:145–152.

Discussion



- In vivo, in a rabbit model of filtration surgery, its potential to significantly delay postoperative scarring has also been demonstrated.

Grisanti S, Diestelhorst M, Heimann K, Krieglstein G. Cellular photoablation to control postoperative fibrosis in a rabbit model of filtration surgery. Br J Ophthalmol 1999; 83:1353–1359.

Discussion



- The efficacy of the photodynamic effect was clinically represented by a functioning filtering bleb with a reduced IOP level (which was maximum in the first week maintained till end of 2nd week and start to rise again by 3rd week and these results were comparable to results of MMC group).

Discussion



- MMC group achieved the highest % of success over the follow-up period.
- Trabeculectomy alone resulted in the highest % of failure. The difference between the groups as regards IOP reduction was marked. Thus, combining trabeculectomy with adjunctive BCECF-AM or MMC makes the procedure more efficient.

Discussion



- The clinical safety and tolerability of photodynamic therapy was represented by no signs of local toxicity or intraocular inflammation, and the lack of any adverse.
- No severe complications as avascularity of the filtering bleb, long lasting hypotony, blebitis, uveitis, phthisis, or endophthalmitis were seen in any of the eyes included in this study.

Discussion



- Though the applied carboxyfluorescein, as a lipophilic drug, could easily penetrate into adjacent superficial ocular tissues, no conjunctival or corneal-epithelial defect was observed in any eye postoperatively.
- The dye was applied preoperatively, subconjunctivally, and the tissue is irradiated before preparing the artificial fistula. Therefore, it is unlikely for carboxyfluorescein to penetrate into the eye.

Discussion



- As a consequence and as already proved by histological analyses of rabbit eyes treated with carboxyfluorescein, ciliary body toxicity was excluded.

Conclusion



Conclusion

- BCECF-AM treated eyes demonstrated favorable bleb morphology and histology.
- The stable density of goblet cells combined with modest fibroblast proliferation and collagen deposition suggest that the use of BCECF-AM may allow for the formation of thicker, more stable blebs.
- Use of BCECF-AM could allow for combination therapy with MMC or 5-FU at lower doses with decreasing the incidence of MMC related complications.

Conclusion

Cellular photoablation using BCECF-AM is a safe and effective wound modulating method to control postoperative fibrosis in trabeculectomy. However MMC considered as a more potent adjuvant to trabeculectomy than BCECF-AM in promoting IOP reduction.

اليوم العلمى لقسم طب وجراحة العيون

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بمركز تدريب وتطوير التعليم
امام مبنى مستشفى عين شمس الجامعى

